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Rome, date

THE DIRECTOR
OF THE DIVISION

(signature)

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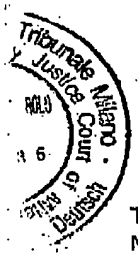


FORM A
DUTY STAMP

TO THE MINISTRY OF INDUSTRY COMMERCE AND HANDICRAFT
Main Patent Office - ROME
Patent Application for Industrial Invention, filing of reserves,
advanced opening to public inspection

A. Applicant (1) G.S. Limited Liability Company
1) Name FIDIA ADVANCED BIOPOLYMERS S.r.l.
Residence BRINDISI code 01510440744

2) Name G.S.
Residence code

B. APPLICANT'S REPRESENTATIVE BEFORE M.P.O.
Surname, name Fiscal code
Name of the corresponding office
Address city zip code province

C. ELECTED DOMICILE OF THE ADDRESSEE FIDIA ADVANCED BIOPOLYMERS S.r.l.
Address Via Ponte della Fabbrica 3/A city ABANO TERME code 35031 prov PD

D. TITLE proposed class, (sec./cl./ucl.) group/subgroup

Sulphated hyaluronic acid and the derivatives thereof, covalently bound to synthetic polymers for the preparation of biomaterials and for the coating of biomedical objects in the fields of health care and surgery

ADVANCED OPENING TO PUBLIC INSPECTION yes___ no__X_
in presence of amendment request: date no. of ref.:

E. NAMED INVENTORS

surname, name	surname, name
1) BARBUCCI Rolando	3) MAGNANI Agnese
2) CONSUMI Marco	4) CALLEGARO Lanfranco

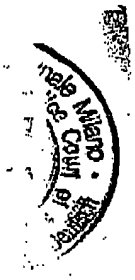
F. PRIORITY
Country or Exhibition Type of Priority Appln. No. Appln. date Encl(yes/res)

1) NONE

2)

G. CENTRE FOR COLLECTING MICROORGANISMS'CULTURES, denomination

H. SPECIAL NOTES
None



ENCLOSED DOCUMENTS

			RESERVES DISSOLUTION
Specimen No.			date No. of ref.
Doc. 1) 1 prov.	no. sheets 21	abstract with main drawing, spec. and claims (compulsory 1 copy)	
Doc. 2) 0 prov.		0 drawing (compulsory if cited in description, 1 copy)	
Doc. 3) 0 res.		power of attorney or reference attorney	
Doc. 4) 0 res.		designation of inventor	
Doc. 5) 0 res.		priority document with Italian translation	comparison single priority
Doc. 6) 0 res.		authorisation or assignment deed	
Doc. 7) 0 res.		complete name of the applicant	

8) PAYMENT RECEIPT OF LIT. 565.000.= compulsory
filled in on 29.08.1996

The applicant's signature Company's seal

follows yes/no NO
We required certified copy of the present deed yes/no YES

PROVINCIAL OFFICE OF INDUSTRY COMMERCE HANDICRAFT OF PADOVA code 28
FILING CERTIFICATE Application no. PD 98 A 000037 Reg. A
The year 1998 the 25th day of the month of February

The above mentioned applicant(s) has(have) presented to me undersigned the present application consisting of no. 00 additional sheets for the grant of the above patent.

I. DIFFERENT NOTES OF THE RECORDING OFFICER
none

THE DEPOSITER
(signature)

THE RECORDING OFFICER
(signature)
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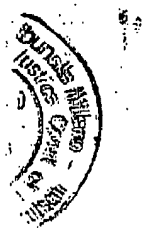
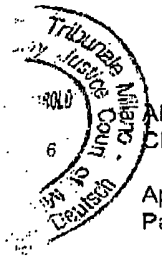




FORM A

ABSTRACT OF THE INVENTION TOGETHER WITH MAIN DRAWING, SPECIFICATIONS AND CLAIMS

Application No. PD98A000037 Reg.A — Filing date 25.02.1998
Patent No. — Date of grant

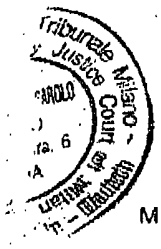
APPLICANT (I)
Name FIDIA ADVANCED BIOPOLYMERS S.r.l.
Residence Brindisi

D. TITLE

Sulphated hyaluronic acid and the derivatives thereof, covalently bound to synthetic polymers for the preparation of biomaterials and for the coating of biomedical objects in the fields of health care and surgery

L. ABSTRACT

The present invention concerns new, haemocompatible derivatives constituted by a polyurethane covalently bound to sulphated hyaluronic acid or its sulphated derivatives, and the process for their preparation.



M. DRAWING

Description of a patent application for an industrial invention entitled "Sulphated hyaluronic acid and the derivatives thereof, covalently bound to synthetic polymers for the preparation of biomaterials and for the coating of biomedical objects in the fields of health care and surgery" by Fidia Advanced Biopolymers S.r.l. with its headquarters in via De' Carpentieri 3, 72100 – Brindisi, Italy, in the person of its President and Legal Representative, Dr Lanfranco Callegaro.

Inventors: Rolando BARBUCCI
Marco CONSUMI
Agnese MAGNANI
Lanfranco CALLEGARO

Filed on 25th February 1998 under No. PD98A000037

**** ****

SUBJECT OF THE INVENTION

The present invention concerns new, haemocompatible derivatives constituted by a polyurethane covalently bound to sulphated hyaluronic acid or its sulphated derivatives, and the process for their preparation.

FIELD OF THE INVENTION

Considerable efforts have been made over the last few decades in the synthesis and surface modification of constantly new classes of polymers, in order to provide bio- and haemocompatible materials for use in surgery.

Polyurethanes are widely used in biomedical applications because of their good mechanical and haemocompatible properties.

In order to enhance the latter property, molecules able to inhibit the coagulative process have been bound to the surface of polyurethane.

These substances are usually chosen from among those which can prevent platelet adhesion and aggregation, or block coagulation factors.

Heparin is one of the modifying agents used and this can be bound to the polymer surface by both ionic (US 4,944,767) and covalent (W. Marconi et al., Makromol. Chem. 194, 1347-1356, 1993) bonds.

These bonds can be achieved once the polymer surface has been chemically modified by introducing reactive groups such as carboxy, hydroxy and amino groups.

Other modifying agents with anticoagulant properties are O-sulphated hyaluronic acid and its O-sulphated derivatives, prepared according to the method described in the international patent application by the Applicant, No. WO 95/25751.

Lastly, of considerable importance are N-sulphated hyaluronic acid and its N-sulphated derivatives, optionally salified, wherein the glucosamines are partially N-sulphated or partially N-sulphated and partially or totally O-sulphated in position 6, as described in Italian patent application by the Applicant No. PD97A000064 filed on 4.4.97.

These sulphated derivatives have anticoagulative, non-thrombogenic, antiviral and anti-inflammatory properties and it has been demonstrated that they inhibit platelet adhesion, aggregation and activation.

One of the main drawbacks to the use of heparin is its high degradation rate on account of the enzyme heparinase, which limits its possible applications in fields of surgery such as cardiovascular surgery, which may call for the implant of devices where the absence of thrombogenicity must be guaranteed for lengthy periods.

The sulphated derivatives, on the other hand, prove particularly advantageous in resisting the enzyme hyaluronidase, and they therefore ensure anticoagulant activity for far longer than heparin (G. Abatangelo et al., Biomaterials 18, 1997, 1411-1415).

However, the above derivatives, as they stand, cannot be processed in the form of biomaterials.

The aim of the present invention is to provide new derivatives with a high degree of haemocompatibility, constituted by a polyurethane bound covalently to sulphated hyaluronic acid or its sulphated derivatives. Said biomaterial maintains its mechanical characteristics (resistance to wear and tear, bending, elasticity etc.), the stability of polyurethane and its anticoagulant activities, inhibiting platelet adhesion, activation and aggregation and the sulphated derivatives' resistance to hyaluronidase.

Moreover, the derivatives according to the present invention present the considerable advantage of being easily mobilised on the polymer surface of biomedical objects, exploiting their solubility in organic solvents. Indeed, the surface of an object made of polymer material can be treated with the organic solution of the derivative triggering solubilization of the outer layers of the polymer and, due to the subsequent evaporation of the solvent, the derivative adheres to the surface, merging with the polymer material of which the object is made.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes new haemocompatible derivatives constituted by a polyurethane covalently bound to sulphated hyaluronic acid or the sulphated derivatives thereof and the process for their preparation.

The preparation of O-sulphated hyaluronic acid or the O-sulphated derivatives thereof is described in the patent application by the Applicant No. WO95/25751.

By N-sulphated hyaluronic acid and the N-sulphated derivatives thereof, we mean hyaluronic acid or its derivatives wherein the glucosamines are partially N-sulphated or partially N-sulphated and partially or totally O-sulphated in position 6.

Said compounds are prepared by a controlled sulphation reaction of the amino group of the glucosamine of hyaluronic acid, previously N-de-acylated according to the procedure described by P. Shaklee (1984) J. Biochem. 217, 187-197.

By partially 2-N-sulphated and 6-O-sulphated derivatives, we mean products wherein the primary hydroxy function of the same residue, besides the amino group of the glucosamine, is totally or partially involved in the sulphation process, as described in the earlier Italian patent application by the Applicant No. PD97A000064 filed on 4.4.97.

By sulphated derivatives of hyaluronic acid we mean the following esters: the partial or total esters wherein part or all of the carboxy groups are reacted with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series (EP 0216453); the crosslinked esters wherein part or all of the carboxy groups belonging to the D-glucuronic residue are reacted, respectively: i) by the use of condensing agents with the alcoholic functions of the same polysaccharide chain or other chains, generating inner (or lactone) esters and inter-molecular esters (EP 0341745); ii) with poly-alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating crosslinking by means of spacer chains (EP 265116).

The compounds according to the present invention, obtained by covalent bonds between the polyurethane and the sulphated compounds, can be used alone or in association with other natural, synthetic or semisynthetic polymers and/or pharmaceutically active substances for the preparation and coating of biomedical objects.

The pharmaceutically active substances that can be used are, for example, antibiotics, anti-infective, antimicrobial, antiviral, cytostatic, antitumoral, anti-inflammatory and wound healing agents, anaesthetics, cholinergic or adrenergic agonists and antagonists, antithrombotic, anticoagulant, haemostatic, fibrinolytic, thrombolytic agents, proteins and their fragments, peptides, polynucleotides, growth factors, enzymes, vaccines.

Of the natural polymers, it is possible to use, for example, collagen, coprecipitates of collagen and glycosamino glycans, cellulose, polysaccharides in the form of gels such as chitin, chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan, alginic acid or alginates, polymannan or polyglycans, starch and natural gums. The semisynthetic polymers, for example, can be chosen from the group consisting of collagen crosslinked with agents such as aldehydes or precursors of the same, dicarboxylic acid or the halogenides thereof, diamines, derivatives of cellulose, hyaluronic acid, chitin or chitosan, gellan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gum and glycosamino glycans. Lastly, of the synthetic polymers it is possible to use, for example, polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxanes polyphosphazenes, polysulphonic resins, PTFE.

The forms of biomaterial in which the derivatives according to the present invention can be processed are, for example: sponges, films, membranes, threads, tampons, nonwoven fabrics, microspheres, nanospheres, gauzes, gels, guide channels.

The biomaterials thus obtained can be used in the cardiovascular field or in any application involving contact with the blood or with highly vascularised body tissues.

The above biomaterials can be used to advantage in various surgical fields, in internal, osteoarticular, neurological, anastomotic, viscoelastic, ophthalmic, oncological, aesthetic, plastic, otorhinolaryngological, abdominal-pelvic, urogynaecological and cardiovascular surgery, in the prevention of post-surgical adhesions and in the prevention of hypertrophic scarring.

The biomaterials according to the present invention can be used, besides in the surgical field, in haemodialysis, in cardiology, in dermatology, in ophthalmology, in otorhinolaryngology, in dentistry, in gynaecology, in urology and in extracorporeal blood circulation and oxygenation.

The above biomaterials in their various forms can also be used to advantage as cell culture supports, such as for mesechymal cells or mature cells to obtain connective, glandular and nerve tissue.

These compounds can also be used in the processes of preparation and coating of objects for use both in the medical field and in industry, forming new biological characteristics on the surfaces of materials used as supports.

The objects that can be prepared or coated are, for example, catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone and cardiovascular replacements, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreases and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for the regeneration of cells and tissues, supports for peptides, proteins and antibodies.

The process of preparation of the derivatives according to the present invention involves the use of suitable compounds able to create a bridge between the polyurethane and sulphated hyaluronic acid or its sulphated derivatives.

Indeed, these last are bound to polyurethane, for example, by hexamethylenediisocyanate (HMDI) or by N-N'dicyclohexylcarbodiimide (DCC) and bromoacetic acid.

EXAMPLE 1

Process for the preparation of the material constituted by polyurethane covalently bound to sulphated hyaluronic acid (PU-HAsulph) by N-N'dicyclohexylcarbodiimide (DCC) and bromoacetic acid

Thirty ml of a 10% (w/v) solution of DMF are supplemented with 1.5 g of dicyclohexylcarbodiimide while agitating.

Once the dicyclohexylcarbodiimide has dissolved, 1.8 g of bromoacetic acid dissolved in a minimal quantity of DMF are added drop by drop.

After 30-40 minutes the solution is filtered to separate it from the white dicyclohexylurea precipitate. Two hundred mg of sulphated hyaluronic acid are added (molecular weight 200 Kda and degree of sulphation 3.5), bicarbonate of soda and this is left to react for 24 hours while being agitated at a temperature of 25°C.

If any precipitate has formed, the solution is filtered again then cast in Petri dishes.

We report hereafter the infra-red spectra (Fig. 1) of the sulphated hyaluronic acid with a degree of sulphation of 3.5, of the PU-HAsulph derivative in its dry and wet forms.

The PU-HAsulph material in its dry state presents the spectrum typical of polyurethane not modified with sulphated hyaluronic acid, while in its wet state, peaks of between 3600 and 2800 cm^{-1} and at 1654cm^{-1} can be seen relative to the functional groups of sulphated hyaluronic acid.

EXAMPLE 2

Process for the preparation of the material constituted by polyurethane covalently bound to sulphated hyaluronic acid (PU-HAsulph) by hexamethylenediisocyanate (HMDI)

Three hundred mg of sulphated hyaluronic acid with a degree of sulphation of 3.5, wherein the carboxyl is in its acid form, is dissolved in a minimal quantity of DMF.

Once solubilisation is complete, the solution is placed in a flask containing 200 µl of HMDI under agitation and in an inert atmosphere.

Thirty minutes later, 10 ml of a 10% (w/v) solution of polyurethane is added.

The solution is left under agitation and in an inert atmosphere at a temperature of 45-50°C for 3 days. It is then cast in Petri dishes.

EXAMPLE 3

Process for the preparation of the material constituted by polyurethane covalently bound to sulphated hyaluronic acid (PU-HAsulph) by N-N'dicylcohexylcarbodiimide (DCC) and bromoacetic acid

Thirty ml of a 10% (w/v) solution of polyurethane in DMF is supplemented with 1.5 g of dicyclohexylcarbodiimide under agitation.

Once the dicyclohexylcarbodiimide has dissolved, 1.8 g of bromoacetic acid dissolved in a minimal quantity of DMF is added drop by drop.

Thirty to forty minutes later, the solution is filtered to separate it from the white dicyclohexylurea precipitate. It is supplemented with 200 mg of N-sulphated hyaluronic acid (molecular weight 200 Kda and 30% sulphation) bicarbonate of soda and it is left to react for 24 hours under agitation at a temperature of 25°C.

It is filtered again and then cast in Petri dishes.

These methods are not, however, limiting to the aims of the invention and any variation that would appear evident to an expert in the field comes within the scope of the invention.

EXAMPLE 4

Test of platelet adhesion on the material obtained according to example 1

Blood was drawn from a healthy, non-smoking donor who had taken no drugs for a fortnight before. Platelet-rich plasma (PRP) was obtained by centrifuging the whole blood at 250 rpm for 25 minutes at room temperature.

One ml of PRP was placed in contact with each sample (0.5 cm x 0.5 cm) of the test polymer and these were then left for 3 hours at room temperature in order to favour platelet adhesion. The samples were then washed in PBS (phosphate buffer solution) to remove any platelets which had not adhered to the surface, and then incubated in a solution of gluteraldehyde at 2.5% (v/v) in 100 mM sodium cacodylate for 30 seconds. Subsequently, the films were washed in cacodylate of sodium, 100mM, for 30 seconds, rinsed in distilled water and left in the first dehydrating solution (70% v/v of ethanol in distilled water) for 15 minutes. The samples were then transferred to the second dehydrating solution (90% v/v of ethanol in distilled water) for 15 minutes and lastly in absolute ethanol for another 15 minutes.

All the samples were then dehydrated in a vacuum for 12 hours, metallized with gold and analysed with a scanning electron microscope (SEM) (Figures 2, 3 and 4).

As can be seen from figures 2, 3 and 4, the surface of the material is morphologically irregular and characterised by the presence of numerous slits of varying sizes. Despite these irregularities, 90% of the material presents no phenomena of platelet adhesion. Only on the remaining 10% of the surface can the presence of platelets be observed, which in some cases form small clusters while in others they appear to maintain their individual character even though they have lost the discoid shape typical of non-activated platelets, and they have extruded pseudopods with which they cling to the surface.

EXAMPLE 5

Thrombin time measured using the material obtained according to example 1

The ability of the new derivative according to the present invention to increase blood coagulation time is measured by the thrombin time test conducted with a coagulometer. An assessment is made of the time it takes to transform fibrinogen into fibrin after the addition of an excess of thrombin in a blood sample in the presence of the polymer. A result of over 120 seconds is no longer significant.

The results are reported in the following table:

Table 1

SAMPLES	THROMBIN TIME (seconds)
Control (polystyrene)	12.1 ± 0.9
PU	12.5 ± 0.4
PU-HAsulph air side (Ø 0.8 cm)*	25.4 ± 1.5
PU-HAsulph glass side (Ø 0.8 cm)*	>120
PU-HAsulph (0.8 cm x 0.5 cm) #	26.2 ± 3.8

Key:

PU = polyurethane

PU-HAsulph = polyurethane covalently bound to sulphated hyaluronic acid

* = thrombin time determined directly on material after incubation at 37°C for 10 minutes

\# =thrombin time determined on plasma after contact with material for 10 minutes at 37°C

The table shows that the anticoagulant activity occurs on the side of the film which is in contact with the glass because the polar environment causes the sulphated hyaluronic acid group to be exposed on the surface, while different results are observed on the side which is in contact with the air.

The invention being thus described, it is clear that these methods can be modified in various ways. Such modifications are not to be considered as divergences from the spirit and purpose of the invention and any modification which would appear evident to an expert in the field comes within the scope of the following claims:

CLAIMS

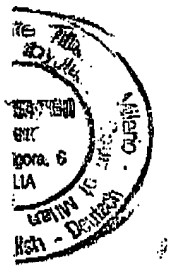
1. Haemocompatible derivatives constituted by a polyurethane covalently bound to sulphated hyaluronic acid or the sulphated derivatives thereof, optionally in association with natural, synthetic, semisynthetic polymers and/or pharmaceutically active substances.
2. Haemocompatible derivatives according to claim 1, wherein the hyaluronic acid and the derivatives thereof are sulphated on the hydroxy group.
3. Haemocompatible derivatives according to claim 1, wherein the glucosamines of the hyaluronic acid or the derivatives thereof are partially N-sulphated or partially N-sulphated and partially or totally O-sulphated in position 6.
4. Haemocompatible derivatives according to claim 1, wherein the term hyaluronic acid derivatives means the partial or total esters wherein a part or all of the carboxy groups are reacted with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series.
5. Haemocompatible derivatives according to claim 1, wherein the term hyaluronic acid derivatives means the crosslinked esters wherein a part or all of the carboxy groups belonging to the D-glucuronic residue are reacted, respectively: i) by the use of condensing agents, with the alcoholic functions of the same polysaccharide chain or other chains, generating inner (or lactonic) esters and inter-molecular esters; ii) with poly-alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating crosslinking by means of spacer chains.
6. Haemocompatible derivatives according to claim 1, wherein the pharmaceutically active substances are antibiotics, antiinfective, antimicrobial, antiviral, cytostatic, antitumoral, anti-inflammatory, wound healing agents, anaesthetics, cholinergic or adrenergic agonists and antagonists, antithrombotic, anticoagulant, haemostatic, fibrinolytic, thrombolytic agents, proteins and their fragments, peptides, polynucleotides, growth factors, enzymes, vaccines.
7. Hamocompatible derivatives according to claim 1, wherein the natural polymers are collagen, coprecipitates of collagen and glycosamino glycans, cellulose, polysaccharides in the form of gels such as agarose, xanthane, gellan, alginic acid or the alginates, polymannan or polyglycans, starch, natural gums.
8. Haemocompatible derivatives according to claim 1, wherein the semisynthetic polymers are collagen crosslinked with agents, such as aldehydes or precursors of the same, dicarboxylic acids or their halogenides, diamines, cellulose derivatives, derivatives of hyaluronic acid, chitin or chitosan, gellan,

xanthane, pectin or pectic acid, of polyglycans, of polymannan, agar, agarose, natural gum, glycosamino glycans.

9. Haemocompatible derivatives according to claim 1, wherein the synthetic polymers are polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxan, polyphosphazenes, polysulphonic resins, PTFE.
10. Haemocompatible derivatives according to claims 1-9, for the preparation or coating of biomedical objects.
11. Haemocompatible derivatives according to claim 10, wherein the biomedical objects are catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone replacements and cardiovascular prostheses, contact lenses, blood oxygenators, artificial kidneys, hearts pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and the regeneration of cells and tissues, supports for peptides, proteins, antibodies.
12. Haemocompatible derivatives according to claims 1-9, for the preparation of biomaterials in the form of sponges, films, membranes, threads, tampons, nonwoven fabrics, microspheres, nanospheres, gauzes, gels, guide channels.
13. Haemocompatible biomaterials constituted by a polyurethane covalently bound to sulphated hyaluronic acid or the sulphated derivatives thereof, optionally in association with natural, synthetic, semisynthetic polymers and/or pharmaceutically active substances.
14. Biomaterials according to claim 13, wherein the pharmaceutically active substances are antibiotics, antiinfective, antimicrobial, antiviral, cytostatic, antitumoral, anti-inflammatory, wound healing agents, anaesthetics, cholinergic or adrenergic agonists or antagonists, antithrombotic, anticoagulant, haemostatic, fibrinolytic, thrombolytic agents, proteins or their fragments, peptides, polynucleotides, growth factors, enzymes, vaccines.
15. Biomaterials according to claim 13, wherein the natural polymers are collagen, collagen coprecipitates and glycosamino glycans, cellulose, polysaccharides in the form of gels such as chitin, chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan, alginic acid or the alginates, polymannan or polyglycans, starch, natural gums.
16. Biomaterials according to claim 13, wherein the semisynthetic polymers are collagen crosslinked with agents such as aldehydes or precursors of the same, dicarboxylic acids or their halogenides, diamines, derivatives of cellulose, hyaluronic acid, chitin or chitosan, gellan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gum, glycosamino glycans.
17. Biomaterials according to claim 13, wherein the synthetic polymers are polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxanes, polyphosphazenes, polysulphonic resins, PTFE.
18. Biomaterials according to claims 13-17, in the form of sponges, films, membranes, threads, tampons, nonwoven fabrics, microspheres, nanospheres, gauzes, gels, guide channels.
19. Use of the haemocompatible derivatives according to claims 1-9 for the preparation or coating of biomedical objects.
20. Use of the haemocompatible derivatives according to claim 19, wherein the biomedical objects are catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin

such as cardiac valves from pigs, artificial tendons, bone replacements or cardiovascular prostheses, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreas and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for cell and tissue regeneration, supports for peptides, proteins, antibodies.

21. Use of the haemocompatible derivatives according to claims 1-9, for the preparation of biomaterials in the form of sponges films, membranes, threads, tampons, nonwoven fabrics, microspheres, nanospheres, gauzes, gel, guide channels.
22. Use of the haemocompatible derivatives according to claims 1-12 in surgery.
23. Use of the haemocompatible derivatives according to claim 22, where surgery is to be taken to mean internal, osteoarticular, neurological, anastomotic, viscoelastic, ophthalmic, oncological, plastic, aesthetic, otorhinolaryngological, abdominal-pelvic, urogynaecological, cardiovascular surgery, in the prevention of post-surgical adhesions and in the prevention of hypertrophic scarring.
24. Use of the haemocompatible derivatives according to claims 1-12, in haemodialysis, in cardiology, in dermatology, in ophthalmology, in otorhinolaryngology, in dentistry, in gynaecology, in urology, in extracorporeal blood circulation and oxygenation.
25. Use of the haemocompatible derivatives according to claims 1-12, for the preparation of supports for cell growth.
26. Process for the preparation of the biomaterials constituted by polyurethane covalently bound with sulphated hyaluronic acid or the sulphated derivatives thereof including the following steps:
    a) reaction of the polyurethane with bromoacetic acid in the presence of N,N'-dicyclohexylcarbodiimide
    b) subsequent reaction with sulphated hyaluronic acid or with the sulphated derivatives thereof
27. Process for the preparation of biomaterials constituted by polyurethane covalently bound with sulphated hyaluronic acid or the sulphated derivatives thereof, including reaction with hexamethylenediisocyanate.



DECLARATION UNDER 37 CFR 1.68

I, Giovanna Luisa Sarolo, declare

That I reside at Via Podgora 6, Milan, Italy;

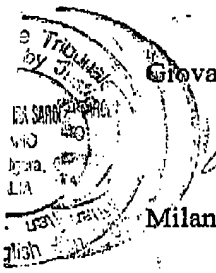
That I am familiar with the Italian and English languages;

That I am a Sworn Translator, appointed by the Court of Milan, Italy;

That I have prepared the attached translation of the Italian Patent Application No. **PD98A000037** filed on **25 February 1998** with the title: "Sulphated hyaluronic acid and sulphated derivatives thereof covalently bound to polyurethanes, and the process for their preparation", said Italian language document being already filed at WIPO during the PCT procedure.

That the attached translation is complete and accurate and fairly reflects the meaning and content of said Italian language document.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.



Giovanna Luisa SAROLO

Milan, ITALY, 8 February 2007